

SOP#104: Tissue Collection Protocol for DNA Analyses

Purpose: The following are recommendations for the collection and preservation of tissue in RNAlater or ethanol or swab samples for use in downstream molecular analyses.

Materials:


Tissue Collection:	Swab Collection:
Nitrile or latex gloves	Nitrile or latex gloves
Forceps, surgical scissors, single use disposable biopsy punches (e.g., Miltex) and/or scalpel	Sterile swabs (1 per sample) in sterile packaging e.g., Puritan HydraFlock swabs, 30 mm breakpoint (VWR, Cat# 10124-664)
50% (v/v) Bleach solution e.g., Javex	Small Ziploc freezer bag for swab handle discards
Distilled water	
Kimwipes/ Clean paper towels	
1.5 mL RNAlater (Ambion; Cat#AM7021) in a 2 mL prelabelled screwtop vial (1 per sample) OR 1.5 mL 95% molecular grade ethanol in a 2 mL prelabelled screwtop vial (1 per sample)	2 mL prelabelled screwtop vial with cap hinge (1 per sample, Sarstedt, Cat# 72.694.217)
Box to store vials	Box to store vials
Black permanent marker (alcohol resistant)	Black permanent marker (alcohol resistant)
Cooler with ice (if possible)	Cooler with ice (if possible)
Sample collection checklist	Sample collection checklist
Pen for completing the checklist	Pen for completing the checklist

Important Notes and Considerations:

1. Ensure that all required collection, transport, and possession permits are in place prior to collection.
2. Nitrile or latex gloves should be worn to avoid sample and specimen contamination.
3. Ensure that the surgical implements or swab only contacts the desired specimen.
4. If not using single-use sterile implements, all implements should be soaked in a 50% (v/v) bleach solution for 30 sec, then rinsed with copious amounts of distilled water (freshly opened bottled water) and wiped with Kimwipes in between each tissue sample collected. It is very important to completely rinse off the bleach so that the tissue is not compromised.
5. Only one tissue-specific sample from a single individual should be placed in each screw-cap tube. Never combine different tissues or individuals in the same collection tube to prevent cross-contamination. For consistency, each tissue type should ideally be sampled from the same region across the animals examined (e.g., the same region of tail muscle or dorsal fin).
6. If you notice crystals in the RNAlater, warm the tube with warm water to 37°C or in your hands and shake to dissolve them before using.
7. The presence of a small air space in the collection tubes is important to protect against burst tubes if they are frozen. However, it is important to keep the tissue fully immersed in preservative solution during shipping and storage.
8. Sample tubes should be clearly prelabelled with a black permanent marker and the sample checklist form should be filled out with the exact same sample name as appears on the tube.

9. If sending tissue samples preserved in 95% non-denatured ethanol, ensure that the dangerous goods paperwork is completed as this is a dangerous good. RNAlater is NOT a dangerous good and requires no additional paperwork.

Tissue Collection Method with RNAlater:

1. Using approved permits, protocols, and procedures and wearing gloves, remove the tissue and immediately place it in a prelabelled screw top tube containing sufficient RNAlater at ambient temperature. The tissue should be no bigger than the size of the eraser on the tip of a pencil (approximately 5 mm in width by 10 mm in length) and placed in at least 5 volumes of RNAlater per 1 volume of tissue. 
2. Ensure that the tissue sample is fully immersed in the RNAlater.
3. Put the tube containing the tissue sample in the storage box and keep in an upright position in the cooler.
4. Collect additional samples as needed.
5. When sampling is complete, **transfer the samples to a refrigerator (4°C) and store upright for at least 24 h.** This is to allow permeation of the tissue with RNAlater and must be performed before it is frozen for storage. **This minimum time is very important.**
6. After incubation at 4°C for at least 24 h and within a month, transfer vials to -20°C in a NOT frost-free freezer for longer term storage and in preparation for shipping.
7. If conditions do not allow for refrigeration right away, store at room temperature for no longer than a day before transferring to 4°C and subsequently to -20°C as in step 6.
8. Proceed to shipping instructions.

Tissue Collection Method with 95% ethanol:

1. Using approved permits, protocols, and procedures and wearing gloves, remove the tissue and immediately place it in a prelabelled tube containing 95% molecular grade ethanol at ambient temperature. The tissue should be no bigger than the size of the eraser on the tip of a pencil (approximately 5 mm in width by 10 mm in length).
2. Ensure that the tissue sample is fully immersed in the ethanol.
3. Store at room temperature, in the refrigerator (4°C), or in the freezer (-20°C).
4. Proceed to shipping instructions. Note that ethanol is a dangerous good.

Swab Collection Method:

1. Wearing gloves, remove swab from packaging.
2. Swab specimen for 10 seconds, rotate the swab to ensure the entire swab contacts the specimen.
3. Allow the swab to air dry briefly.
4. Place into a 2 mL screwtop vial and break the swab tip (at the 30 mm breakpoint) so it fits completely in the vial. Close the vial securely and place the broken off handle into the discard bag for later proper disposal.
5. Put the tube containing the tissue sample in the storage box and keep in an upright position in the cooler.
6. Collect additional samples as needed.
7. When sampling is complete, transfer vials to -20°C in a NOT frost-free freezer for longer term storage and in preparation for shipping.
8. Proceed to shipping instructions.

Shipping Instructions:

1. For shipping, tissue samples immersed in *RNAlater* or the swabs should be placed frozen on precooled blue ice packs. Tissue samples immersed in ethanol can be sent at room temperature however make sure that the appropriate dangerous goods shipping paperwork is in place before shipping.
2. For shipping, make certain that all required paperwork is completed and ship samples early in the work week (Monday or Tuesday) to ensure that the samples are not stranded in transit over the weekend.
3. Below is the shipping address for the Helbing Lab:

Helbing Lab c/o Lab Coordinator
Science Stores
Petch Building Room 168
University of Victoria
3800 Finnerty Road
Victoria BC V8P 5C2
CANADA
(250) 721-7086

4. Email the name of the courier company and the shipping tracking number to the Lab Coordinator along with the completed digital copy of the Sample Collection Checklist.